AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph bridging pages 1 and 2 with the following amended paragraph:

Erythropoietin is ~~a~~ an acidic glycoprotein hormone of approximately 34,000 daltons. Naturally occurring erythropoietin is produced by the liver during fetal life and by the kidney in response to hypoxia (e.g., red blood cell loss due to anemia) and regulates red blood cell growth and differentiation through interaction with its cognate cellular receptor cells into erythrocytes. It is essential for regulating levels of red blood cells in blood circulation of adults and stimulates the production of red blood cells in bone marrow. Anemia is a consequence of renal failure to produce erythropoietin. Recombinant erythropoietin produced by genetic engineering techniques involving the expression of a protein product from a host cell transformed with the gene encoding erythropoietin has been found to be effective when used in the treatment of anemia resulting from chronic renal failure. Wild type, or naturally-occurring, erythropoietin is defined herein to include recombinant erythropoietin (Jacobs, *K., et al., Nature, 313:806-813* (1985)), or naturally-occurring erythropoietin which has been isolated and purified from blood (Miyake, T., et al., J. Biol. Chem., 252:5558-5564 (1977)) or sheep plasma (Goldwasser, E., et al., Proc. Natl. Acad. Sci. U.S.A., 68:697-698 (1971)), or chemically synthesized erythropoietin which can be produced using techniques well known to those of skill in the art. Human erythropoietin is a 166 amino acid polypeptide that exists naturally as a monomer (Lin, F-K., et al., Proc. Natl. Acad. Sci. USA 82:7580-7584 (1985)). The tertiary structure of erythropoietin as an isolated protein and in a complex with its receptor has been reported (Syed RS, et al., Nature [1998] 395:511-6; Cheetham JC, Nat Struct Biol. [1998] 5:861-6). The identification, cloning, and expression of genes encoding erythropoietin are described in U.S. patent 4,703,008. A description of the purification of recombinant erythropoietin from cell medium that supported the growth of mammalian cells containing recombinant erythropoietin plasmids for example, is included in U.S. patent 4,667,016. The expression and recovery of biologically active recombinant erythropoietin from a mammalian cell containing the erythropoietin gene on a recombinant plasmid has, made available quantities of erythropoietin suitable for therapeutic applications. In

addition, knowledge of the gene sequence and the availability of larger quantities of purified protein has led to a better understanding of the mode of action of this protein.
Several forms of anemia, including those associated with renal failure, HIV infection, blood loss and chronic disease can be treated with this hematopoietic growth factor. Erythropoietin is typically administered by intravenous or subcutaneous injection three times weekly at a dose of approximately 25-100 U/kg.

On page 5, please replace the paragraph starting with "Therapeutic fusion proteins" and ending with "(WO 96/08570, US 5,541,087)." with the following amended paragraph:

Therapeutic fusion proteins have also been constructed using the Fc domain ~~to~~ to incorporate functions such as Fc receptor binding, protein A binding, complement fixation and placental transfer which all reside in the Fc proteins of immunoglobulins. For example, the Fc region of an IgG1 antibody has been fused to the N-terminal end of CD30-L, a molecule which binds CD30 receptors expressed on Hodgkin's Disease tumor cells, anaplastic lymphoma cells, T-cell leukemia cells and other malignant cell types (U.S. Patent No. 5,480,981). Furthermore, it has been reported in 1996 that efficient expression and secretion of certain non-mutant target proteins can be achieved by expression of fusion proteins comprising an Fc portion of an immunoglobulin and said target proteins followed by proteolytic cleavage of the target protein (WO 96/08570, US 5,541,087).

Please replace the paragraphs beginning at line 10 of page 22 and ending at line 17 of page 24 with the following amended paragraphs:

Sequence Information

The following DNA and amino acid sequences were used in this invention.

The coding sequence for mature EPO, using modified codons to optimize translation and including bases at the 5' end comprising the SmaI site is given in ~~Seq. Id. No. 1.~~ SEQ ID NO:1

SEQ ID NO:-1

(Small characters indicate base differences from the human EPO coding sequence that are predicted to increase expression but not change protein sequence.-)

CCCGGGtGCCCCACCACGCCTCATCTGTGACAGCCGAGTgCTGGAGAGGTACCTCTTG
GAGGCCAAGGAGGCCGAGAATATCACGACcGGCTGTGCTGAACACTGCAGCTTGAA
TGAGAAcATCACcGTgCCtGACACCAAAGTgAATTTCTATGCCTGGAAGAGGATGGAG
GTtGGcCAGCAGGCCGTAGAAGTgTGGCAGGGCCTGGCCCTGCTGTCGGAAGCTGTCC
TGCGGGGCCAGGCCCTGTTGGTCAACTCTTCCCAGCCGTGGGAGCCCCTGCAaCTGC
ATGTGGATAAAGCCGTgAGTGGCCTTCGCAGCCTCACCACTCTGCTTCGGGCTCTGgG
AGCCCAGAAGGAAGCCATCTCCCCTCCAGATGCGGCCTCAGCTGCTCCcCTCCGcAC
AATCACTGCTGACACTTTCCGCAAACTCTTCCGAGTCTACTCCAATTTCCTCCGGGGA
AAGCTGAAGCTGTACACAGGGGAGGCCTgcCGGACAGGGGACAGATGActcgag

SEQ ID NO:-2 The mature EPO protein sequence (one-letter code)

APPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQ
AVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKE
AISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDR

Oligonucleotides used to construct a fusion of normally glycosylated EPO to the C-terminus of an Fc region.

SEQ ID NO:3 ~~SEQ ID 3:~~ *Oligo 1*

CCGGGtGCCCCACCACGCCTCATCTGTGACAGCCGAGTgCTGGAGAGGTA

CC

SEQ ID NO:4 ~~SEQ ID 4:~~ *Oligo 2*

TCTTGGAGGCCAAGGAGGCCGAGAATATCACGACcGGCTGTGCTGAACA

SEQ ID NO:5 ~~SEQ ID 5:~~ *Oligo 3*

CTGCAGCTTGAATGAGAAcATCACcGTgCCtGACACCAAAGTgAATTTCTAT

SEQ ID NO:6 ~~SEQ ID 6:~~ *Oligo 4*

GCCTGGAAGAGGATGGAGGTtGGcCAGCAGGCCGTAGAAGTgTGGCAG

SEQ ID NO:7 ~~SEQ ID 7:~~*Oligo 5*

GGCCTGGCCCTGCTGTCGGAAGCTGTCCTGCGGGGCCAGGCCCTGTTGGTC

SEQ ID NO:8 ~~SEQ ID 8:~~*Oligo 6*

AACTCTTCCCAGCCGTGGGAGCCCCTGCAaCTGCATGTGGATAAAGCCG

SEQ ID NO:9 ~~SEQ ID 9:~~ *Oligo 7*

TgAGTGGCCTTCGCAGCCTCACCACTCTGCTTCGGGCTCTGgGAGCCCAGAA

SEQ ID NO:10 ~~SEQ ID 10:~~ *Oligo 8*

GGAAGCCATCTCCCCTCCAGATGCGGCCTCAGCTGCTCCcCTCCGcAC

SEQ ID NO:11 ~~SEQ ID 11:~~ *Oligo 9*

AATCACTGCTGACACTTTCCGCAAACTCTTCCGAGTCTACTCCAATTTCCTCC

SEQ ID NO:12 ~~SEQ ID 12:~~ *Oligo 10*

GGGGAAAGCTGAAGCTGTACACAGGGGAGGCCTgcCGGACAGGGGACAGATGActcgag

Mutagenesis of glycosylation sites:

SEQ ID NO:13 ~~SEQ ID 13:~~ Oligo 11 (oligo 2')

tc ttggaggcca aggaggccga gcagatcacg acgggctgtg ctgaaca

TCTTGGAGGCCAAGGAGGCCGAG**CAG**ATCACGACcGGCTGTGCTGAACA

SEQ ID NO:14 ~~SEQ ID 14:~~ *Oligo 12* (oligo 3')

CTGCAGCTTGAATGAG**CAG**ATCACcGTgCCtGACACCAAAGTgAATTTCTAT

SEQ ID NO:15 ~~SEQ ID 15:~~ *Oligo 13* (oligo 6')

CAGTCTTCCCAGCCGTGGGAGCCCCTGCAaCTGCATGTGGATAAAGCCG

SEQ ID NO:16 ~~SEQ ID 16:~~ *Oligo 14* (oligo 8')

GGAAGCCATCTCCCCTCCAGATGCGGCC**GCA**GCTGCTCCcCTCCGcAC

SEQ ID NO:17 ~~SEQ ID 17:~~

Human IgG1 Fc region – mature protein coding sequence

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:18 ~~SEQ ID 18:~~

Human IgG2 constant region – mature protein coding sequence (CH1, hinge, CH2, and CH3 regions)

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSSGLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTVER
KCCVECPPCP APPVAGPSVFLFPPKPKDTL MISRTPEVTC VVVDVSHEDP
EVQFNWYVDG VEVHNAKTKP REEQFNSTFRVVSVLTVVHQ DWLNGKEYKC
KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKNQVSLTCLVKG
FYPSDIAVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK

Please replace the paragraph bridging pages 24 and 25 and starting with "The protein according to" and ending with "standard approaches." with the following amended paragraph:

The protein according to SEQ ID NO:2 ~~SEQ ID 2~~ does not have the N-terminal lysine residue of the mature protein. The synthesized DNA was engineered to have an XmaI-compatible overhang at the 5' end and an XhoI-compatible overhang at the 3' end. An alternative sequence was constructed with mutations in the four EPO glycosylation sites: $Asn_{24} \rightarrow$Gln, $Asn_{38} \rightarrow$ Gln, $Asn_{83} \rightarrow$ Gln, and $Ser_{126} \rightarrow$ Ala. The 500 base-pair DNA was cloned and sequence analysis confirmed that it encodes the mature human EPO without additional undesired mutations.

The expression vector pdCs-Fc-EPO was constructed as follows. The XmaI-XhoI restriction fragment containing the human EPO cDNA was ligated to the XmaI-XhoI fragment of the pdCs-Fc vector according to Lo *et al.* [Protein Engineering (1998) 11:495]. The resultant vector, pdCs-Fc-EPO, was used to transfect mammalian cells for the expression of Fc-EPO. This vector expresses the human immunoglobulin gamma1 chain Fc region. A second set of Fc-EPO vectors were constructed in which the gamma1 chain Fc region was replaced with an Fc region derived from human gamma2.

The Fc protein moiety also usually contains a glycosylation site. This site may be optionally changed to a non-glycosylated sequence by standard approaches.

Please replace the paragraph bridging pages 29 and 30 and starting with "Example 7" and ending with "mutations described above.." with the following amended paragraph:

Example 7

Site-directed mutagenesis of unglycosylated EPO

Mutations that increase the activity of unglycosylated EPO are introduced into the Fc-unglycosylated EPO fusion protein as follows. A DNA sequence encoding unglycosylated EPO is constructed as described in Example 1, except that one pair of oligonucleotides is replaced with a corresponding pair of oligonucleotides encoding a portion of EPO with one or more altered amino acids. For example, to introduce the change Asn147Ala, the oligonucleotide AATCACTGCTGACACTTTCCGCAAACTCTTCCGAGTCTACTCCGCATTCCTCC (SEQ ID NO:27) is used instead of oligo 9 (SEQ ID NO:11) ~~(SEQ ID 11)~~, along with a correspondingly altered reverse-complement oligonucleotide.

The following mutations are introduced by this procedure: $Gly_{101}Ala$, $Arg_{143}Ala$, $Ser_{146}Ala$, and $Asn_{147}Ala$. These mutations most likely have the effect of increasing the activity of Fc-EPO by increasing its affinity for the EPO receptor. As another example, Gln_{65} is mutated to an amino acid that has a smaller and/or more hydrophobic side chain. The effect of this mutation is to increase the fraction of Fc-EPO that is active. This effect is pronounced when mutations in the region of amino acids 114 to 130 are also present.

In other versions, cysteine residues are inserted and removed by substitution as described in Example 13. The resulting protein is more stable and more efficiently expressed, especially when combined with the mutations described above.

On page 37, please replace the paragraph starting with "For example" and ending with "amino acids alone" with the following amended paragraphs:

For example, the following set of experiments with controls is performed. Human Fc-EPO, human Fc-EPO (Cys_{29}-Cys_{88}), human EPO, and human EPO (Cys_{29}-Cys_{88}) are cleaved with trypsin in both reducing and non-reducing conditions. These eight samples are analyzed by mass ~~specrometry~~ spectrometry. Trypsinized non-reduced human Fc-EPO (Cys_{29}-Cys_{88}) and human EPO (Cys_{29}-Cys_{88}) each give a peak with a high molecular weight, corresponding to EAENITTGCAEGPSLNENITVPDTK (SEQ ID NO:28) + GQALLVNSSQPCEPLQLHVDK (SEQ ID NO:29) with two N-linked glycosylations. Because of its large size and heterogeneity due to the presence of two N-glycosylations, this peak is easily distinguished from the other peaks. This peak is not found in reduced samples or in samples derived from non-mutant human EPO or non-mutant human Fc-EPO. As a further diagnostic test, samples are incubated with N-glycanase before treatment with trypsin.
In the samples treated with N-glycanase, the peak corresponding to EAENITTGCAEGPSLNENITVPDTK (SEQ ID NO:28, corresponding to pos. 21 – 45, SEQ ID NO:2 ~~Seq. Id. No. 2~~) +
GQALLVNSSQPCEPLQLHVDK (SEQ ID NO:29, corresponding to pos. 77 – 97, SEQ ID NO:2 ~~Seq. Id. No. 2~~) is shifted to the size predicted by the molecular weights of the amino acids alone.